A sampling protocol for the endangered freshwater fish, Oxleyan Pygmy Perch *Nannoperca oxleyana* Whitley

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ABSTRACT

The conservation of the Oxleyan Pygmy Perch *Nannoperca oxleyana* would benefit from the adoption of a standardised, non-destructive sampling protocol that effectively and efficiently detects the presence, and quantifies the relative abundance, of extant populations. The objectives of this study were to: (i) quantify the minimum number of traps required to obtain precise relative abundance estimates, (ii) assess the effectiveness and efficiency of various trapping regimes, and (iii) compare the relative detectability and short-term mortality rates of trapping, seine netting and backpack electrofishing. Previous survey data were utilised and augmented with field experiments. Ten traps provided relatively precise estimates of relative abundance. Unbaited and baited traps set for 30 and 60 minutes detected the species on all occasions, whereas traps set for 15 minutes did not. Positive correlations were found between set time and both overall (fish/trap) and standardised (fish/trap/minute) catch rates, although only the former relationship was significant. The addition of bait to traps did not significantly affect catch rates. Trapping, seine netting and electrofishing detection rates were 88%, 71% and 83%, respectively. Associated mortality rates were 10%, 55% and 0%, respectively. The deployment of multiple gear types increased the likelihood of detecting *N. oxleyana*. A sampling protocol is recommended that includes saturating sites with unbaited traps set for at least 30 minutes and sampling with a backpack electrofisher. Seine netting should be reserved for situations where an electrofisher is unavailable or non-deployable.

Key words: endangered fish, *Nannoperca oxleyana,* trap, seine, electrofishing, efficiency, effectiveness, mortality

Introduction

The Oxleyan Pygmy Perch *Nannoperca oxleyana* Whitley is a freshwater percichthyid fish (Fig. 1) distributed along a narrow belt of lowland, coastal habitat in mid-eastern Australia (Pusey *et al.* 2004). This small, bottom-dwelling, habitat specialist grows to approximately 60 mm in length and occupies shallow, swampy regions of dystrophic, acidic freshwater streams, lakes and swamps draining through sandy, wallum (*Banksia* dominated heath) ecosystems (Fig. 2) (Arthington 1996; Knight in press). Large expanses

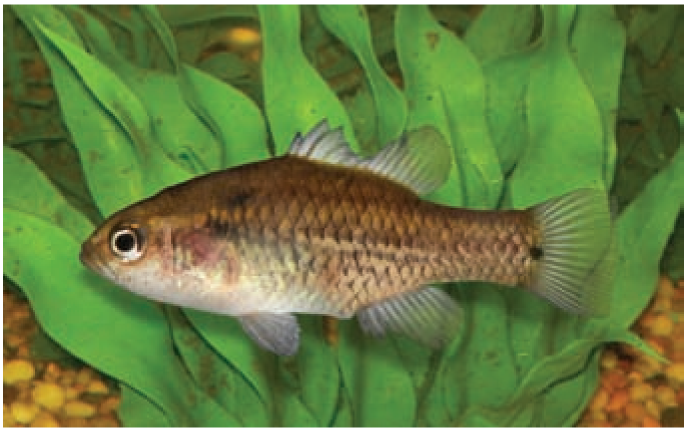

Figure 1. Oxleyan pygmy perch *Nannoperca oxleyana.* Large mature female, 50 mm total length. Photo: DPI

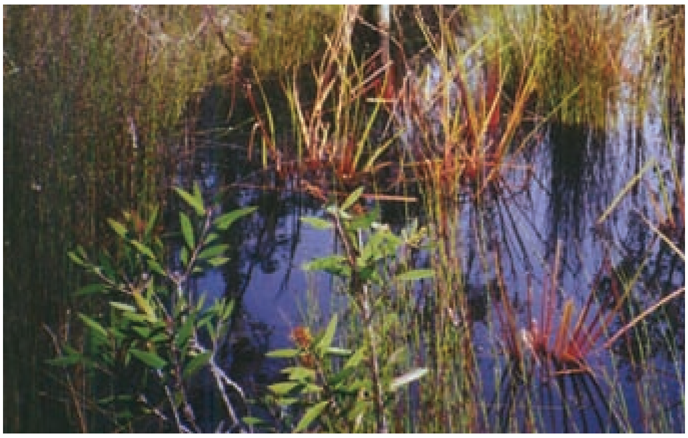

Figure 2. Typical swampy habitat of *N. oxleyana* within a dystrophic, acidic, freshwater stream near Evans Head, New South Wales. The 'red' emergent macrophyte *Philydrum lanuginosum* typically dominates the littoral zone and provides important habitat for the fish. Photo: J. Knight

of this habitat have been destroyed or degraded by human activities. As a result, *N. oxleyana* is considered endangered and the remaining populations are typically fragmented and patchily distributed. A recovery plan has been prepared to guide long-term conservation and management programs (DPI 2005).

In recent years, there has been an increase in the number of studies aimed at documenting the locations and relative sizes of remaining *N. oxleyana* populations. This information is fundamental to effective management, primarily because it assists with decisions regarding environmental impact and the need for habitat protection and provides a basis for developing and implementing future surveys to determine the effectiveness of recovery actions. In reviewing the surveys undertaken to date, Knight (2000) noted inconsistencies in the documented spatio-temporal distribution patterns of *N. oxleyana*, which in part may be attributed to the relative effectiveness of various sampling regimes to capture the species.

The main techniques used to assess distributions have included sampling with small collapsible traps set either unbaited or baited for between 15 and 90 minutes, as well as active gear such as seine nets and backpack electrofishers (e.g. Arthington 1996; Bishop 1999; Esdaile 2000; Knight in press). Dip netting has also been used on occasion (e.g. Leggett 1990).

As with all fish sampling methods, each of the above gear types may be spatially or temporally biased towards sampling particular species of fish (Hayes *et al.* 1996; Hubert 1996). When designing a study, it is important to select sampling gear types that are the least biased and provide the most accurate data possible (Willis and Murphy 1996). The ability to accurately detect a rare species such as *N. oxleyana* may, however, be hindered by its reduced distribution and abundance (Green and Young 1993; Jackson and Harvey 1997). Given that the conservation management of a threatened species such as *N. oxleyana* relies heavily upon robust scientific data, it is imperative that a standardised sampling protocol is adopted that effectively detects presence and absence patterns and gives reliable estimates of relative abundance.

Spatial and temporal comparisons of population size are best derived by standardised sampling methods. To date, trapping has been the primary technique used to study the ecology of *N. oxleyana* populations (Arthington and Marshall 1993; Arthington 1996; Knight 2000, in press). When examining changes in relative abundance through estimates of catch per unit effort (CPUE), an optimal level of precision is required (Kingsford 1998). Concomitantly, there is also a need to avoid excessive sampling by establishing efficient and cost effective protocols (Angermeiser and Smogor 1995; Growns *et al.* 1996).

Sampling protocols for threatened species should aim to utilise the least invasive methods and avoid techniques that may be destructive to both the target population and its habitat (Kelsch and Shields 1996; Neilson 1998; Craig 2006). While trapping, seining and electrofishing have been used to catch *N. oxleyana* in a range of habitats, the associated fishing mortality of each gear type has not been quantified. All gear has the potential to cause short- or long-term injuries that could negatively affect health, behaviour, growth, and reproduction or ultimately cause death (Kelsch and Shields 1996; Snyder 2003). Techniques such as seine netting are also capable of disturbing fish habitats (Ivantsoff *et al.* 1988; J. Knight, personal observations).

Given the above, this study aimed to develop a standardised, non-destructive sampling protocol that effectively and efficiently targeted *N. oxleyana*. The specific objectives were to: (i) quantify the minimum number of traps required to obtain precise relative abundance estimates, (ii) assess the effectiveness and efficiency of various trapping regimes to capture *N. oxleyana*, and (iii) compare the relative detectability and short-term mortality rates of trapping, seine netting and backpack electrofishing. These analyses were restricted to *N. oxleyana* because an adequate data set on sympatric species was not available.

Methods

Gear Specifications

Fish traps were similar to those used in previous studies of *N. oxleyana* and other small-bodied fishes (Arthington and Marshall 1993; Arthington 1996; Bishop 1999; Balcombe and Closs 2000; Knight 2000, in press). Traps measured 250 x 250 x 450 mm and consisted of 3 mm nylon mesh covering a rectangular, collapsible wire frame with an open, inverted funnel at each end with 40 mm openings. The seine net, which was specifically designed to sample the narrow, overgrown drainage systems typical of the wallum ecosystems, was constructed from 3 mm, knotless (i.e. non-abrasive), polyester nylon mesh, measured 4 x 1.5 m, had a centred pocket 1 m in length, a weighted foot-rope and a floating head-rope. Electrofishing was done with a Smith-Root model 12B, battery powered, backpack electrofisher, using a 280 mm diameter aluminium anode ring attached to a fibreglass handle, and a rat-tail cable cathode. Depending on conductivity, electrical output ranged from 200 to 500 volts of pulsed direct current (DC) at a fixed pulse rate of 60 Hz. Affected fish were collected using a dip net constructed with the same mesh as that used for the seine net.

Trapping regimes and precision

The minimum number of traps required to gather precise estimates of relative abundance was determined through analysis of the relationship between the number of traps and sampling precision (standard error, S.E.) (Andrew and Mapstone 1987; Kingsford 1998). CPUE data were derived from five sets of 18-40 traps set unbaited for 30 minutes in two lentic and one lotic system near Evans Head, New South Wales (NSW), Australia (29°07'S. 153°26'E). For each given number of traps within each of the five data sets, the S.E. of the accumulated catch was calculated. The mean S.E. and its variance (± S.E.) were computed and plotted against the number of replicate traps as a negative decay curve.

A trapping field experiment was undertaken to determine the effect of bait and set time on detection rates and relative abundance estimates of *N. oxleyana*. The experiment was run over four consecutive days from 17 to

20 August 2004 in a 1.5 hectare swamp near Evans Head. This site was selected due to its homogeneous habitat and because it was known to support a large population of *N. oxleyana* (mean CPUE of 1.1 fish/trap/0.5 hour versus a mode for NSW *N. oxleyana* sites of 0.1 fish/trap/0.5 hour; J. Knight, unpublished data). Low abundances of other fish species inhabited the swamp and were rarely caught. The study site had a uniform depth of 0.5 m and a sand substratum covered by a thick layer of detritus. The emergent macrophyte *Philydrum lanuginosum* and submerged aquatic moss *Sphagnum falcatulum* dominated the littoral zone and provided the majority of fish habitat. The uniformity of habitat characteristics presumably provided a relatively uniform distribution of fish within the swamp, which would minimise variability between experimental replicates (Montgomery 2005). During the experiments, water temperatures ranged from 13 to 18°C and conductivity was approximately 132μs/cm^{-1}.

Treatments included unbaited and baited traps set for 15, 30 and 60 minutes. Unbaited traps were set on days 1 and 3 and traps baited with a combination of one 3 g cracker biscuit and 20 g of chicken pellets were set on days 2 and 4. Within a day, three sites spread evenly throughout the swamp were sampled simultaneously on three trial occasions. Within a site, during each 'within-day' trial, a group of 10 traps (sample size derived from precision estimates, see results) were set at 1.5-2.0 m intervals on the substrate for a standardised set time of either 15, 30 or 60 minutes. To minimise any site variability, set time was systematically rotated among sites for each sampling trial. This systematic rotation was also included across experimental days so that sites were not repeatedly sampled with the same set time for a given replicate. At the end of the 15 and 30 minute set time treatments, the traps were collected and fish removed, identified and counted. Fish were transferred to a bucket in the shade and the emptied traps were then reset to standardise overall sampling effort within and among sites. Any additional fish captured in these traps were not included in the study. After 60 minutes, all traps were pulled, fish caught in traps set for the full 60 minutes were counted and then all fish collected during the trial were released at their place of capture.

Data from the trapping field experiment were analysed with the software program Multilevel Modelling for Windows (MLwiN 1.1; Rasbash *et al.* 2000) to examine the treatment effects on overall (fish per trap) and standardised (catch per trap per minute, CPUE) catch rates. A Multilevel Poisson Regression Model was chosen as it dealt specifically with hierarchical random factors, used a flexible estimation method (2nd Order Partial Quasi Likelihood approximation) and efficiently analysed the positively skewed count data by employing a log-link function for the Poisson distribution. An Extra Poisson model provided an appropriate fit of the over-dispersed data (i.e. variance > mean). Two independent models were tested for systematic variation within a hierarchical sampling structure, which included sites, days within sites, trials within days, and traps within trials. The models predicted the log probability of the dependent variables, fish/trap and CPUE, varying in relation to bait type, set time, and combinations of these predictors. Given that a Poisson model deals intrinsically with count data, estimates for the CPUE model were adjusted for per minute of set time by including an offset in the model. This is analogous to modelling CPUE data. The model outputs included a partial coefficient and the S.E. of each predictor. The partial coefficient represents the relationship between the variances of the predictor and dependent variable not explained by the other predictors. The significance of an effect parameter, which estimated the difference between a pair of predictors, was determined by the Wald Statistic, as expressed by the equation: Wald = coefficient/S.E. The Wald Statistic was squared and compared to the Chi-squared distribution with one degree of freedom so that statistics ≥ 3.84 were two tailed significant to $p<0.05$. For each pair of significant predictors, tests for significant differences were made using pairwise comparisons. *p*-values were adjusted for multiple comparisons using the Bonferroni procedure. Model estimated mean catch rates ± 95% joint confidence intervals were calculated for each significant predictor. The model was parameterised using treatment contrasts with the first level of a predictor being taken as the intercept. For further details, refer to Snijders and Bosker (1999).

Gear related detection rates

Comparisons of the relative efficacy of trapping, seine netting and backpack electrofishing to detect *N. oxleyana* were made by examining survey data collected by Knight (2000, in press, unpublished data). Species detection was defined as the percentage of sampled water bodies known to support the species in which at least one specimen was detected, with a detection success of 75% or greater considered high (Reynolds 1996). Depending on site conditions, between nine and 40 unbaited traps were set for 15 to 30 minutes at 1.5-2.0 m intervals on the substrate within a range of microhabitats including aquatic vegetation, steep or undercut banks fringed with overhanging vegetation and open waters. Backpack electrofishing and seine netting were also used within similar microhabitats. Site conditions (e.g. conductivity, depth, presence of woody debris and other obstructions) generally dictated if active techniques could be deployed and which technique was used. In sites suitable for active sampling, conditions also affected the amount of sampling effort, thereby restricting sampling to 2-3 seine shots or 10 minutes of electrofishing. Restricted sampling effort is often an inherent problem when sampling freshwater fish and has been noted by other authors (e.g. Chessman 2006).

Gear related mortality

A field experiment was carried out to assess the short-term *N. oxleyana* mortality rates of trapping, seining and electrofishing from 12-16 August 2004 in the same water body used for the trapping study. Sampling involved setting unbaited traps for 30 minutes, and undertaking seine net shots covering 20 m^2 and two minute electrofishing shots (500v, 60Hz pulsed DC). Sampling was repeated until 20 *N. oxleyana* per technique were collected. After capture, fish were quickly examined for signs of any external injuries before being randomly transferred to one of two sealed traps per technique giving 10 fish per trap. Traps were wrapped in 70% shade cloth to aid in the protection of fish from direct sunlight and potential predators, labelled with the gear type used and submerged in the swamp. After 96 hours, the numbers of live and dead fish were recorded and the total lengths of a random subsample of 26 live and dead fish were measured to the nearest 0.1 mm. Live fish were again assessed for injuries before being released.

A Kolmogorov-Smirnov two-sample test was used to test for any significant differences between the lengths of live and dead fish.

Results

Trapping regimes and precision

The relationship between the numbers of traps and sampling precision is depicted in Fig. 3. Mean S.E. decreased rapidly from 0.70 for 2 traps to 0.37 for 5 traps, gradually reduced to 0.26 for 10 traps and declined little thereafter. Similarly, variation in mean S.E. decreased and remained relatively constant for 10 or more traps. Hence, a set of 10 traps was considered to provide relatively precise estimates of CPUE, with increases in effort beyond this point only marginally increasing precision.

For the trapping field experiment, the overall minimum and maximum numbers of traps set on each sampling occasion for each treatment that detected *N. oxleyana* is given in Table 1. Regardless of the baiting technique employed, a set time of 60 minutes had the highest minimum and maximum detection rates while 15 minutes had the lowest. Traps set for 30 and 60 minutes caught individuals on all sampling occasions, whereas those set for 15 minutes failed to catch any individuals on two occasions.

Table 1. Overall ranges for the numbers of traps set on each sampling occasion that detected *N. oxleyana.*

Set time (min)	Unbaited traps		Baited traps	
	Min	Max	Min	Max
15	0	4	0	6
30	2	7	1	7
60	5	10	2	8

Positive correlations existed between mean fish/trap and set time for unbaited and baited traps (Fig. 4a) and between mean CPUE and set time for unbaited traps (Fig. 4c). However, mean CPUE peaked for baited traps at 30 minutes before declining slightly at 60 minutes (Fig. 4c). Fish/trap and CPUE varied little between unbaited and baited traps for the first replicate day of each treatment (days 1 and 2 of the experiment) (Fig. 4b and 4d). Similar catches were also recorded for unbaited traps on the second replicate day (day 3). However, the 2nd replicate for baited traps had a greatly reduced catch rate (day 4), possibly as a result of fish exhibiting trap shyness or bait avoidance on the final day of the experiment. Mean fish/trap and CPUE for baited traps set for 30 and 60 minutes was lower on this day, although S.E. remained similar between each bait treatment for a given set time when data were pooled (Fig. 4a and 4c). Consequently, the multilevel modelling results suggested a day effect on catch rates (Table 2), but this random effect was not significant ($\chi^2 = 3.02$, $p>0.05$).

The fish/trap multilevel model initially included set time, bait and the bait-set time interactive terms but no significant effects of the latter two terms on catch rate were found and so these were removed (Table 2). In addition to set time, the extra Poisson variance was also significant ($\chi^2 = 166.56$, $p<0.0001$), thereby indicating that the catch data were over dispersed. This clumping suggests

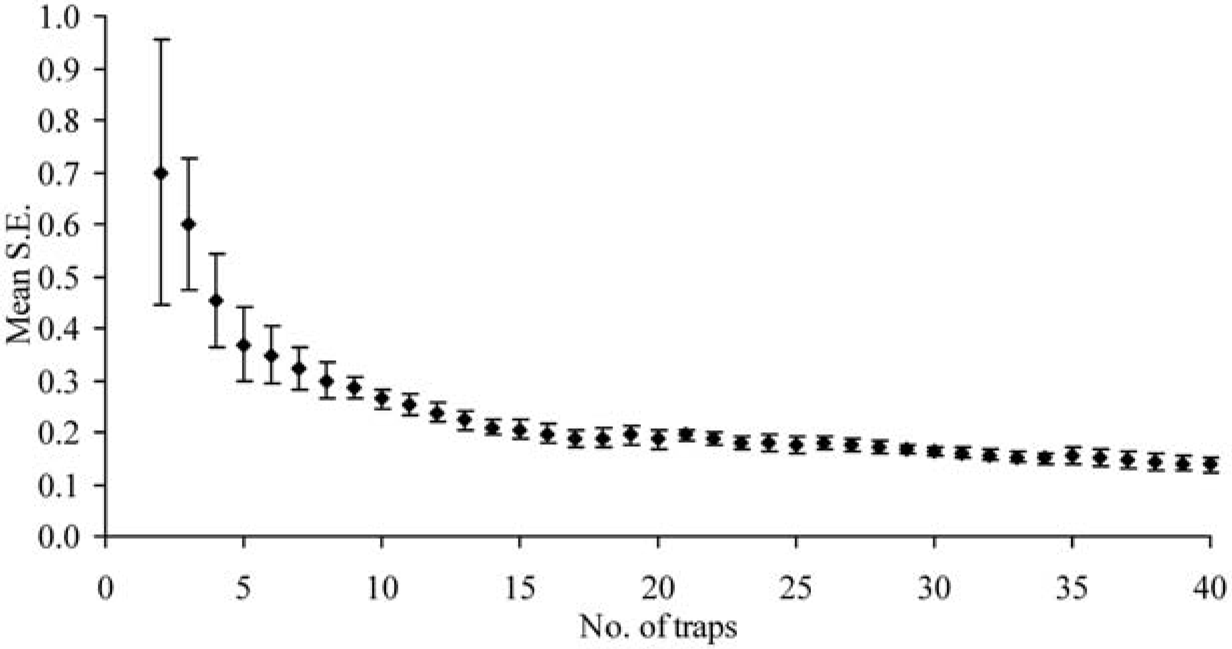

Figure 3. Relationship between the number of traps and sampling precision. Bars represent the variance (± S.E.) of the mean S.E. n = 5.

Table 2. Parameter estimates for the fish/trap multilevel Poisson regression analysis of trap set times.

Effect	Coefficient	S.E.
Fixed effects		
Intercept (15 minutes)	-1.451	0.325
15 vs 30 minutes	0.905	0.266
15 vs 60 minutes	0.308	0.247
Random effects		
Site	0.037	0.170
Day	0.497	0.286
Trial	0.037	0.067
Extra Poisson variance	1.510	0.117

that catches were density dependant. No significant differences were found between CPUE and any of the fixed effects. Coefficients and S.E.s for the random effects were the same as for the fish/trap model.

For the fish/trap model, Bonferroni adjusted multiple comparisons between the three set times were all significantly different (Table 3). As expected from the raw data (Fig. 4a), the model estimated a positive correlation between fish/trap and set time (Table 4). On average, 1.28 *N. oxleyana* were expected to be caught in each trap set for 60 minutes. This estimate was 2.2 and 5.6 times larger than the mean for 30 and 15 minutes, respectively. Likewise, the model estimated mean for the 30 minute treatment was 2.5 times higher than the mean for 15 minutes. Note that the upper and lower joint confidence intervals are not symmetrical as they were derived from logged values (Table 4).

Table 3. Significance test results of set time multiple comparisons derived from analysis of fish/trap data; α = 0.017, Bonferroni adjusted. Wald Statistic = coefficient/S.E.

Set time comparisons	Wald Statistic	Chi-square value	p-value
15 vs 30 minutes	3.402	11.571	<0.001
15 vs 60 minutes	6.858	47.081	<0.0001
30 vs 60 minutes	4.288	18.289	<0.0001

Gear related detection rates

Knight (2000, in press, unpublished data) caught *N. oxleyana* in 55 water bodies. Traps were deployed in 50 of these localities and detected the species on

Table 4. Unlogged estimated mean fish/trap catch rates and joint confidence intervals for each set time treatment.

Set time (min)	Estimated mean fish/trap	Joint confidence interval - 95%	Joint confidence interval + 95%
15	0.23	0.095	0.581
30	0.58	0.265	1.267
60	1.28	0.614	2.646

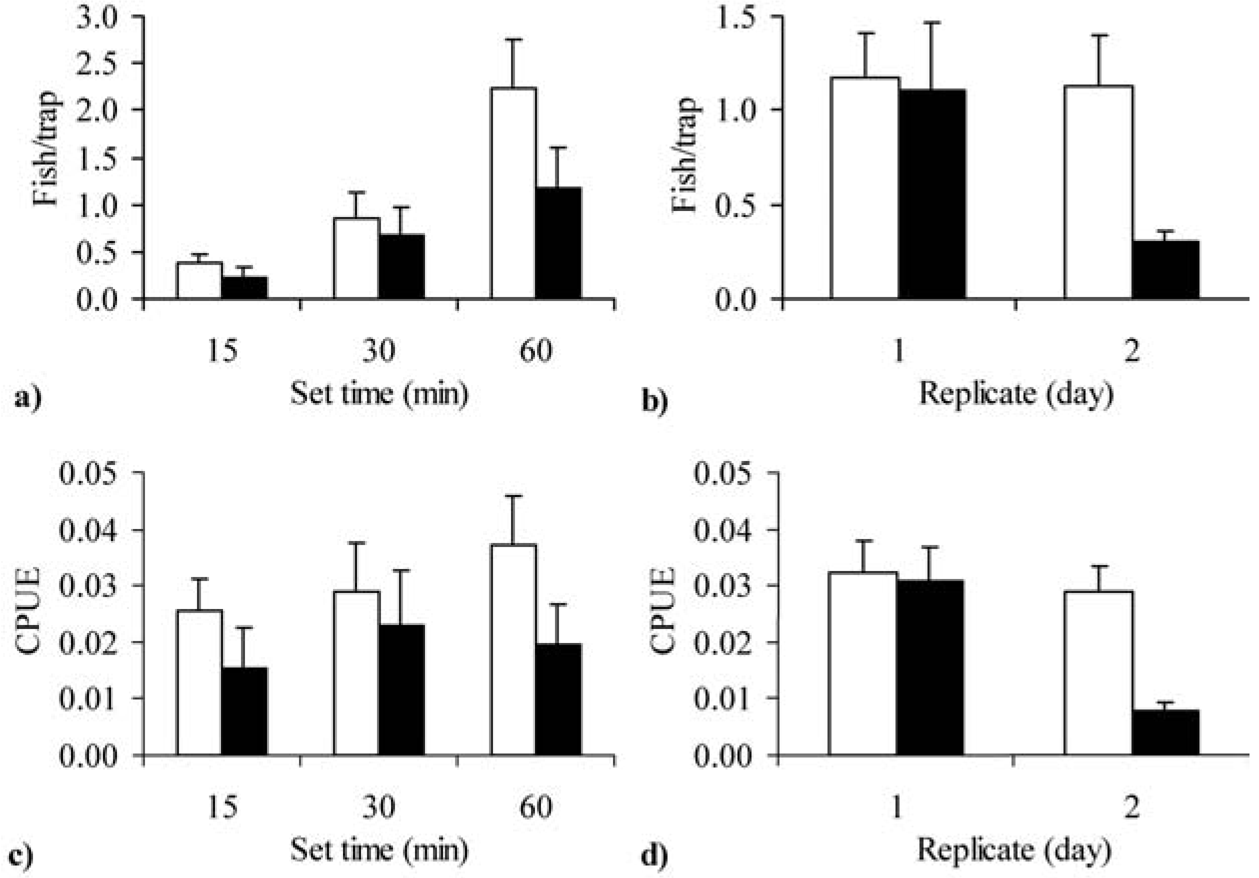

Figure 4. Mean (+ S.E.) fish/trap and CPUE (fish/trap/minute) for unbaited (□) and baited traps (■) for a) and c) the three set times and b) and d) replicate days. Means and S.E. reflect within day trial replication. n = 6 for a) and c), n = 3 for b) and d).

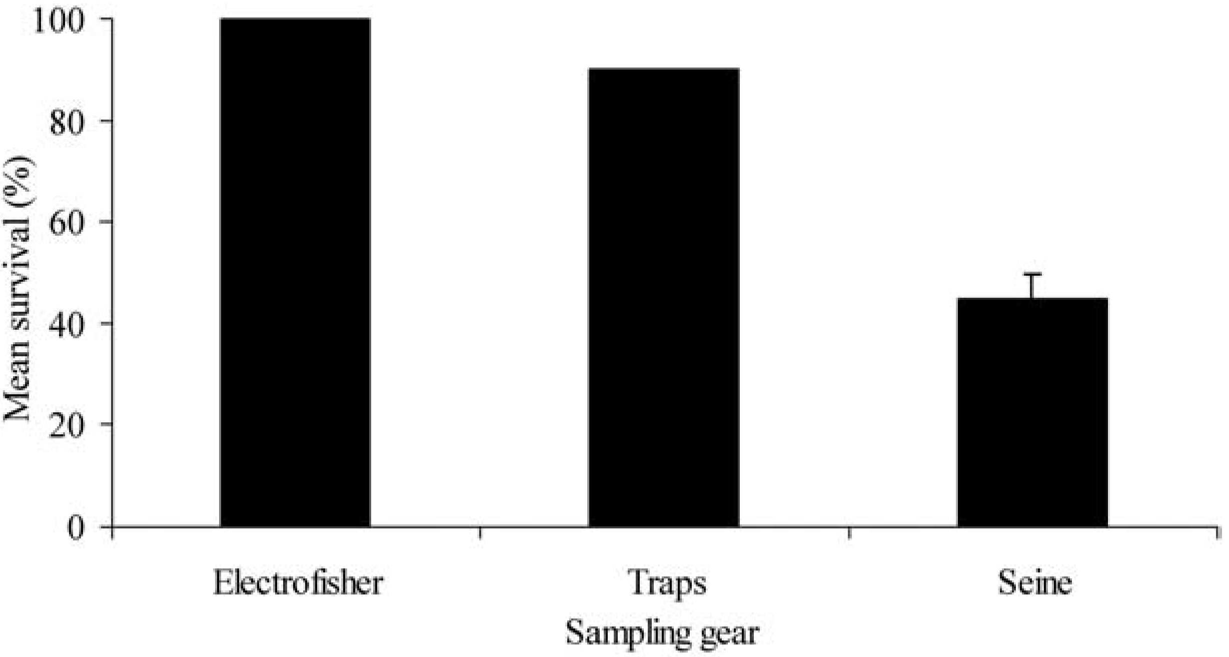

Figure 5. Mean (+ S.E.) survival of *N. oxleyana* captured by backpack electrofishing, trapping and seine netting. n = 2 replicate sealed traps per sampling technique.

88% of sampling occasions (Table 5). Although less regularly utilised, the backpack electrofisher detected fish at a similar rate to trapping, whereas the seine only succeeded in sampling individuals at 71% of the sites in which it was hauled. In total, 38 of the 55 localities inhabited by the species were sampled with multiple gear. When deployed together, both traps and seine nets retained *N. oxleyana* on 61% of sampling occasions, while traps and electrofishing both retained fish on 50% of occasions. However on a number of occasions each technique succeeded in catching individuals while the concomitant gear did not. The majority of the 17 localities not sampled with multiple gear were sampled by trapping alone, with 2 localities sampled solely by backpack electrofishing and 3 localities by dip netting.

Gear related mortality

All 20 *N. oxleyana* caught with the electrofisher and held for 96 hours survived (Fig. 5). The electrical output (500v, 60Hz pulsed DC) induced galvanotaxis (forced swimming) in *N. oxleyana* sufficient enough to allow capture without causing tetany (muscle contraction, rigidity, loss of equilibrium) (see Reynolds 1996). Upon capture and at the time of release, all fish were actively mobile with no visible signs of injury. Similar observations were made for fish caught in traps, although 10% of fish died in each replicate. The mortality rate from seine netting was highest, with a mean of 55% (S.E. ± 5.0) of the fish captured with this technique deceased after 96 h. After capture, most fish appeared to have external injuries including loss of mucus and scales and

Table 5. Effectiveness of sampling gear to detect *N. oxleyana* from 55 inhabited water bodies including the number of localities in which a) each gear was deployed, and b) and c) multiple gears were deployed. Detection rate is defined as the number of localities in which *N. oxleyana* was successfully sampled by a) each gear, b) both gear types deployed, and c) only one of the two gear types deployed. Data derived from surveys by Knight (2000, in press, unpublished data).

Sampling gear comparisons		No. of localities gear deployed	Detection rate Total	%
a) Single gear	Traps	50	44	88
	Seine	28	20	71
	Electrofisher	12	10	83
	Dip net	3	3	100
b) Multiple gear	Traps and seine	28	17	61
	Traps and electrofisher	10	5	50
c) Multiple gear	Only traps successful	38	10	26
	Only seine successful	28	3	11
	Only electrofisher successful	10	3	30

integument abrasions. Only 9 fish were released after 96 h, with 3 displaying external injuries and inhibited swimming ability. There was no significant difference between the total lengths of live and dead fish used in the experiment ($D = 0.308$, $p > 0.1$, n = 13, size range = 18-42 mm).

Discussion

This study has highlighted the effectiveness of using multiple techniques when sampling to detect rare species. The deployment of multiple gear maximises the chances of detecting a species because the selectivity of one particular gear may be offset by that of another (Angermeier and Smogor 1995; Jackson and Harvey 1997). For example, the catching power of passive gear such as traps partially depends on fish behaviour, which in turn may be influenced by environmental parameters like water temperature (Stott 1970; Hubert 1996). Therefore, although *N. oxleyana* is typically prone to capture by traps, during times when fish activity is hindered by extremes in temperature, the species may be more effectively sampled by an active gear such as a backpack electrofisher.

While Knight (2000, in press, unpublished data) used multiple gear where possible, environmental conditions dictated which techniques were used and at times resulted in the deployment of only one gear type. In particular, traps were deployed in 17 sites that precluded the use of active gear. These small, lightweight, collapsible and hence highly portable traps are well suited to sampling small, bottom dwelling, cryptic fishes such as *N. oxleyana*. They are also cost effective, being relatively cheap to purchase and requiring minimal maintenance and effort to deploy (Bloom 1976; Swales 1987; Hubert 1996; Balcombe and Closs 2000). They do however, require a water depth of at least 200 mm to be effective, and are prone to some variability in catch rates. But, as discussed by other authors (e.g. Bagenal 1972; Hubert 1996), the results presented here demonstrate that such variability can be reduced by increasing sampling effort and standardising sampling methods.

This study attempted to standardise trapping methods by investigating differences in detection and catch rates between unbaited and baited traps and among various set times. The addition of bait to a trap did not influence detection rates or significantly affect catch rates. These results support other studies comparing the catch rates of *N. oxleyana* and sympatric species in traps either unbaited or baited with several different fish attractants (Arthington and Marshall 1993; Knight 2000). Unbaited trapping is therefore recommended as the preferred sampling method for future studies, as it is a faster and therefore more efficient method than baiting traps, requires less gear maintenance and eliminates unnecessary, albeit low costs associated with purchasing bait. Baited traps also have the disadvantage that they may create biases in other research such as microhabitat selection studies, as any element of attraction created by the bait may draw fish from other habitats, rather than reflect fish abundance in the habitat being sampled (Culp and Glozier 1989; Arthington 1996; Balcombe and Closs 2000).

The efficiency of a predetermined set time to catch a species largely depends on environmental factors, fish behaviour, and population densities (Swales 1987). The positive correlation between CPUE and set time and the extra Poisson variance indicate that catch rates were density dependent. Hence, it appears that as more fish entered a trap more followed. Although *N. oxleyana* does not exhibit schooling behaviour, fish have been observed to move in pairs or in small groups (Pusey *et al*. 2004).

The significant positive relationship between fish/trap and set time and the non-significant trend in increasing CPUE over time may also be related to the high density of *N. oxleyana* in the swamp with the set times doing little to saturate the traps or deplete the numbers of fish available to enter the traps. Under these circumstances, longer set times may have been required for catch rates to begin to plateau. The density of fish is also likely to have influenced the detection rates of each set time in a similar manner (Swales 1987).

The lack of a significant increase in standardised catch rates over time suggests that 15 minutes is the most cost effective time to set traps. Of particular interest, however, is that, despite the high fish densities, the 15 minute set time failed to detect the species on two occasions. It therefore seems prudent to suggest that traps should be set for a minimum of 30 minutes to maximise the likelihood of detecting *N. oxleyana*. This set time resulted in a high detection rate in targeted surveys by Knight (in press) and located populations that were not detected in previous surveys. As evidenced by the fish/trap data, a 30 minute set time also provided higher overall catches than 15 minutes, which in turn would give a data set with less zeros and greater precision and hence allow for more powerful statistical analysis of spatial and temporal patterns in relative abundances.

The minimum number of 10 traps was found to obtain precise estimates of relative abundances. When undertaking monitoring studies, this may be considered the most cost-effective sampling strategy, as increases in effort beyond this point provided only marginal increases in precision. However, the number or density of traps required to detect *N. oxleyana* within a water body was not examined. Low sampling effort is more likely than greater effort to miss rare species, therefore reducing the accuracy and precision of a sampling strategy and inflating perceived variability in their distribution (Angermeier and Smogor 1995; Jackson and Harvey 1997). Given that failing to detect *N. oxleyana* may lead to inaccurate conclusions regarding its distribution, and negatively affect the conservation and recovery of the species, it is recommended that a large number of traps be deployed. The numbers set should ideally be dictated by the amount of habitat available for sampling. Obviously, practical considerations also play a role, with 40 traps found to be the maximum number that can be carried by a two-person team loaded with a backpack electrofisher, a seine net and other sampling gear when hiking into areas inaccessible by vehicles. Effects of trap density were not experimentally investigated but experience indicates that traps set approximately 1.5-2 m apart effectively sample an area without unnecessarily duplicating effort. Using this criterion in situations where available *N. oxleyana* habitat is limited, at least 10 traps usually can be deployed. Finally, Arthington (1996) recorded higher catch rates of *N. oxleyana* at 1600 hours than at 0800 hours. This information may be useful when planning monitoring programs or small distribution surveys but is difficult to apply when surveying numerous sites within limited time frames.

Although less successful than trapping, the active sampling gear used to survey for *N. oxleyana* also had a moderate to high detection rate. This is despite limited sampling effort associated with restrictive site conditions providing catch data, which may have underestimated or given low precision estimates of the relative effectiveness of the two active gear types. Site conditions also resulted in active gear being deployed in fewer water bodies than the passive gear, thereby illustrating some of constraints of these techniques. The use and effectiveness of each of the active gear types is normally dictated by field conditions and the target species. For example, electrofishing is often more suitable than seining for sampling large fish and in areas obstructed by dense aquatic vegetation or woody debris. Conversely, seining is generally more effective at sampling small fish and may be used in water bodies that are too saline or deep to electrofish (Dauble and Gray 1980; Weaver *et al.* 1993; Onorato *et al.* 1998; J. Knight, personal observations). Dip netting is generally most effective at catching *N. oxleyana* in habitats too shallow or small to adequately sample with other techniques because the species can be quite mobile and evasive (Arthington 1996; J. Knight, personal observations). The high detection rate of fish by backpack electrofishing highlights the susceptibility of this small-bodied fish to this technique and suggests that the electrical output settings (200-500v, 60Hz pulsed DC) used were effective.

The appropriateness of the electrical output is further demonstrated by the behaviour of the target fish. These settings induced galvanotaxis in *N. oxleyana* sufficient enough to allow capture without causing tetany. An electrical waveform that is too intense will induce tetany and may cause injury or death, depending on the size and the species (Reynolds 1996). Similar affects can occur within close proximity to the anode and also depending on the type of electrical current used. As such, the aim of electrofishing is to stimulate a behavioural response in fish that will result in their capture while avoiding injury and minimising stress (Reynolds 1996). The results of this study are similar to those often reported, with mortality being generally uncommon (Snyder 2003, but see Henry *et al.* 2004). If electrofishing results in death, it generally occurs quickly (McMichael 1993; Reynolds 1996; Henry *et al.* 2004) with most minor or moderately injured fish usually surviving and appearing to behave normally (Neilson 1998).

Seine netting is often considered a relatively benign technique that kills few fish (Dauble and Gray 1980; Onorato *et al.* 1998; Kelsch and Shields 1996), although habitat disturbance has been reported (Onorato *et al.* 1998). Dauble and Gray (1980) noted that some mortality did occur as a result of physical injury when rocks were caught in the net. In a similar way, the high mortality and external injury rate recorded in the current study may be at least partially attributed to the amount of organic material collected in the seine. The net dragged macrophytes, sedges and detritus from the soft sandy substrate and accumulated large quantities of aquatic moss *Sphagnum falcatulum*. This debris may have resulted in the injuries observed, including loss of mucus and scales and integument abrasions. The netting used could have contributed to these injuries, but attempts were made to minimise this affect by using knotless (i.e. non-abrasive), polyester nylon mesh. The observed external injuries may have lead to reduced fitness and/or disease and then death (Kelsch and Shields 1996).

The survival rate of fish caught in traps was high although there was a 10% mortality rate. While these fish may have had reduced fitness prior to being captured, it is likely that they sustained injuries by being unintentionally dragged across the abrasive trap mesh when removed. To this end, care should be taken when removing fish. Handling fish with wet hands or gloves and avoiding unnecessary contact should also be considered (Kelsch and Shields 1996; NHMRC 2004). Given that the species is small in size and tends to display more cryptic and evasive behaviours than other sympatric species (e.g. Eleotrids, Melanotaeniids, Poeciliids), traps should also be thoroughly checked as fish are regularly found buried under overlapping seams in the trap mesh or lying motionless in the corners.

It is acknowledged that there may have been additional mortalities or injuries associated with each gear that were not detected in this study. For example, unaccounted mortality could have occurred to fish that actively escaped from a gear, or that died and dropped out of the gear, prior to the catch being removed from the water (Broadhurst *et al.* 2006). Unaccounted delayed mortality may have also resulted as a consequence of seine netting's degradation of the aquatic environment (ICES 2004), particularly given that aquatic vegetation forms an important part of the preferred cover, feeding, breeding and nursery habitats for *N. oxleyana* (Arthington 1996; Knight 2000, unpublished data). Similarly, it is possible that internal electrofishing injuries and associated long term effects on health, behaviour, growth and reproduction may have occurred (Reynolds 1996; Snyder 2003). Elucidation of any additional harmful effects should be the subject of future research, perhaps utilising captive reared specimens or surrogate species of similar size, morphology and behaviour.

In summary, a protocol for surveying *N. oxleyana* populations is recommended whereby sites are saturated with unbaited traps set for at least 30 minutes and concomitantly sampled with backpack electrofishing. When deriving trapping estimates of relative abundance, a total of 10 traps provides the most efficient sampling strategy. Until further information on the effects of electrofishing on *N. oxleyana* are known, the technique should be used with caution and set at the minimum output necessary to be effective. Given the high mortality rate of seine netting and its associated habitat disturbance, this technique should be reserved for situations where an electrofisher is unavailable or non-deployable. As seine netting has been shown to be reasonably effective in detecting *N. oxleyana*, the decision to use the technique should be based on weighing its potential destructiveness against the importance of accurately documenting the species' distribution. Dip netting may supplement this sampling protocol and may be useful in areas unable to be sampled effectively with the primary techniques. Regardless of the sampling method employed, appropriate techniques for the removal, care and handling of fish should be adopted.

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